

## SERUM ALKALINE PHOSPHATASE A PROSPECTIVE BIOMARKER FOR ASSESSMENT OF PROGRESS OF FRACTURE HEALING

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### ABSTRACT

In routine clinical practice, bone healing progress is assessed clinico-radiologically. Furthermore, it is difficult to identify the delayed and non-unions early, sometimes even with advanced imaging techniques. Thus increasing the morbidity of the patients. Serum biomarkers, such as alkaline phosphatase (ALP) a marker of bone formation, may be useful in such cases. Serum ALP levels were quantified at regular intervals in 50 adult patients with fresh closed traumatic diaphyseal fractures of long bones. Regular follow up of these patients was done till either bone union or maximum up to the end of 09 month. Depending on the clinico-radiological outcome, all 50 patients were allocated into 03 groups by the end of follow up; Group A (n = 33): normal union; Group B (n = 11): delayed union and group C (n= 06): Non-union. Mean serum ALP levels followed the same pattern in group A and B, reaching a maximum level at post trauma 6th week. But the mean levels of serum ALP at every selected interval was significantly higher in group A than group B till complete union, later on it was higher in group B. In Group C, mean serum ALP level remained within normal limits throughout the follow-up. So, the determination of serum ALP levels during fracture healing could be an additional tool in predicting fractures at risk of delayed / nonunion, aiding the clinician to prefer the early appropriate intervention at appropriate period.

**KEYWORDS:** Alkaline Phosphatase, Biomarker of Fracture Healing

### INTRODUCTION

Fracture healing is a proliferative physiological process to facilitate the repair of a fracture [1]. This process is characterized by the production of a new organic matrix i.e. osteoid and its subsequent mineralization, thus bridging the gap between two bony fragments. This process of fracture healing should be serially quantifiable / measureable [2]. Till date, there is no clinically validated method to measure healing progression. So a valid measurement for bone union is desired to measure the bony union process. Thus, the values yielded by measurement should be on a continuous numerical scale [3]. However till now, clinicians watch for an end point of complete union without bothering for documenting the values signifying progress of healing [4]. The probability of correct radiological evaluation of the stage of union in fractures of tibia has been shown to be only about fifty percent; thus, the radiographic assessment is not an optimum method to assess the fracture healing, as shown by a study on radiological evaluation of the stage of union in fractures of tibia [5]. None of the present day available measures of union help in the early detection of problems in the bone healing process, resulting into problems of bone union [6]. As the routine methods of diagnosis available may not be able to identify the complications like delayed nonunion at an earlier stage leading to increased morbidity of patients.

Furthermore, it is sometimes hard to differentiate radiologically a delayed union from nonunion at an early stage without the use of more advanced imaging techniques, such as nuclear scintigraphy, which are still available at only few centers in India [7]. So, the assessment of specific bone formation markers, such as ALP at regular intervals may be used in evaluating the progress of fracture healing process [8, 9]. In one study, it has been observed that serum bone ALP and creatinine corrected urinary collagen crosslinks were significantly higher among women who sustained an osteoporotic fracture compared with those women who did not had fracture [10].

In a study, serum ALP activity was found positively correlated with the long bone fractures' healing progress in dogs. Serum phosphorus and calcium changes followed a proportional and inverse pattern to ALP changes in the same study respectively [11]. In another study, the changes in total alkaline phosphatase in femoral and trochantric fractures had been found to be more in trochantric fractures than neck fractures and both fractures expressed similarly for serum ALP post-operatively [12]. With this background, we hypothesised that the serum alkaline phosphatase levels suggest the bone forming activity (responsible for both, bone matrix formation and its mineralization), thus its serial serum estimations will correlate with progression of fracture healing process and may predict the fracture healing outcome earlier. This work was undertaken to evaluate the relationship between serial serum ALP levels and fracture healing outcome.

## MATERIALS AND METHODS

Total 50 patients of age group 18 to 45 years with closed, traumatic, fresh (< 7 days) diaphyseal fractures of long bones managed surgically were included in this study.

### Exclusion Criterion

- Patient with polytrauma
- Pathological fractures
- Pregnant woman
- Immunocompromised patients
- Patients on oral contraceptives and immunosuppressive drugs,
- Patients with uncontrolled diabetes, Hepatobiliary diseases, Inflammatory bowel disease and those who were not willing to give consent for inclusion in the study.

2ml of peripheral venous blood was collected in EDTA vials. Quantitative estimation of serum ALP activity (at pH-10.4, Temp-37°C) was done spectrophotometrically (405nm) using p-Nitrophenylphosphate as a substrate and other reagent provided by BEACON. The sample was processed in standardized manner under set protocol in the department of biochemistry, New Medical College and Hospital, Kota . Serum ALP estimation of each patient was done pre-operatively, 2<sup>nd</sup> post-operative day, at 3week, 6 week, 3month and 6th month.

Gentle clinical examination of the fracture site was done at 6th, 10th week, for the assessment of – skin condition, abnormal mobility, bony tenderness, transmitted movements. Patients were evaluated radiologically at 6<sup>th</sup> and 10th week. Further, management decisions were taken by the co-investigators. Based on the clinico-radiological outcome, patients were divided into 3 groups – Group A:(normal union) clinico-radiological union achieved by the end of 06 months, Group

B: (delayed union) clinicoradiological union not completed by the end of 06 months but completed by the end of 09 months and Group C: (non union) clinico-radiological healing not completed even by the end of 09 months. Clinically union was defined as the stage when the fracture site was painless (no tenderness), motionless (no abnormal mobility); with presence of transmitted movements. Radiologically union was said to be achieved when bony callus was evident on at least 3 cortices in standard AP and Lateral views.

The mean values of serum ALP, serially recorded throughout the follow up period, were compared. Data was estimated on excel sheet and analysed statistically. Quantitative data was summarized in the form of  $MEAN \pm SD$  and results on categorical measurements are presented in Number (%). Serial Serum ALP levels of patients of all the groups were compared by standard error of difference between means. Results on categorical measurements were compared by standard error of difference between proportions. The level of significance was kept 95% for all statistical analysis.

## RESULTS AND DISCUSSIONS

Total 50 patients were enrolled in this study, who were allocated into group A (N = 33), group B (N = 11) and group C (n = 6), depending on the progression, duration and type of bone healing. The average age of group A was 35.4 (range 18-45) years, group B was 40 (range 22-25) years and was 38.2 (range 30.6 – 39.7) years for group C. The difference between the mean ages of these groups was not found to be significant. The mean age of fracture at the time of admission in these groups was 1.6 (range 1-4) days, 1.3 (range 1-2) days and 1.8 (range 1-3) days respectively (the difference between the age of fracture at admission was insignificant). The bony healing occurred at 19.2 (range 18 – 22) weeks in group A and at 30 (range 27 – 33) weeks in group B. The mean serum ALP levels remained within normal limits (45-120 U/L) in all patients at the time of admission. The variation in ALP serum levels followed the same pattern in group A and B, reaching a maximum level at 6th week in both groups{figure 1} (maximum mean of group A 437.8 U/L and of group B 307 U/L). Serum ALP levels after being elevated significantly till 6 weeks,declined to normal range at clinico-radiological union in group A.

In group B mean serum ALP levels were found to be significantly elevated even till 06 months. Till bony union, at every interval, the mean values of serum ALP of group A were significantly higher than that of group B. The changes in mean serum ALP levels in group C were never significant and remained within normal limits throughout follow up.

Fracture healing is a proliferative physiological process to facilitate the repair of a fracture. Earlier clinicians have relied on clinicoradiological evaluation methods but these methods lack objectivity and are not reliable. Bone formation markers as ALP can serve as an accurate, precise, reliable, reproducible, patient – doctor friendly and cost effective method to measure fracture healing objectively.

Normal fracture healing is generated by increasing osteoblastic activity. There is secretion of large quantities of ALP by Osteoblasts, that is involved in the formation of bone matrix and its mineralization [8]. ALP is believed to either increase the concentration of local inorganic phosphate or neutralize inorganic pyrophosphate, which is an inhibitor of hydroxyapatite crystal formation [13-14]. Although serum ALP levels correlate well with the process of fracture healing, the bone isoenzyme of ALP (BALP) is considered more specific marker for bone formation [8]. The inclusion criteria set for this study eliminated the possibility of other ALP isoenzymes being responsible for the significant increases in ALP levels in these enrolled cases.

Delayed union is considered as a fracture that requires more time for complete bone union than the usual but will unite ultimately [16], which is the case of group B (mean union at 30 weeks). Nonunion is defined as the cessation of all reparative processes of fracture healing without bony union [17]. A fracture that at minimum of 9 months post occurrence is not completely healed, and has not shown radiographic progression for 3 months is labeled as nonunion which is the case of group C [18]. The incidence of nonunion of diaphyseal tibial fracture varies from 6 – 41% [16]. In the present study, the incidence of clinico-radiological nonunion at the end of 09 months was 23%. In our study, the serial serum ALP levels were correlated with the clinico-radiological progression of bony fracture healing. Our finding related with ALP level variation during fracture healing were corresponding with that of other studies [12, 19].

In our study, changes in serum ALP level in group A and B paralleled the process of fracture healing as documented by clinico-radiological evidences. Though, in group A, mean ALP returned to normal reference limits by clinico-radiological fracture healing, in group B these levels remained elevated till 06 months, suggestive of ongoing osteoblastic activity in these patients. At 06 months, clinico - radiologically it was not evident that which patients will fall into delayed union and which one will be in nonunion. Waiting 9 months to diagnose and treat a nonunion is unreasonable in modern world. This dilemma raised a question that whether a clinician should wait for radiological signs to settled down to establish a diagnosis of nonunion or one can predict and can intervene early by observing a biomarker, like ALP. We may predict the future outcome of these fractures at as early as 6th week by observing the serum ALP levels.

## CONCLUSIONS

The serial measurement of serum ALP levels can be an additional, useful, reproducible, patient – clinician friendly and cost effective tool in predicting the fractures that are at risk of developing complications like delayed union / nonunion so that clinicians can decide to intervene properly at an earlier point of time decreasing morbidity and improving the outcome.

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## APPENDICES

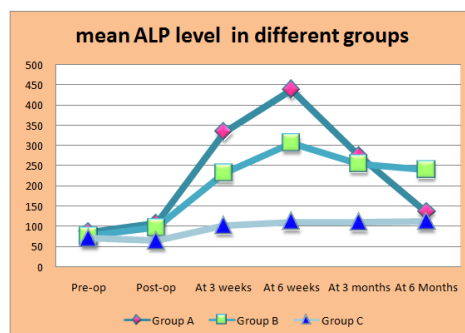


Figure 1: Mean Alp Levels in Different Groups during Follow-Up

